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Molecular Weight of Poliovirus Ribonucleic Acid

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Purified poliovirus single- and double-stranded ribonucleic acids (RNA) were examined by electron microscopy. The length of both molecules was found to be 2.37 μm. The uncorrected sedimentation coefficient for single-stranded RNA is 33*S*, as compared to 27*S* for the RNA of tobacco mosaic virus. It is calculated from these results that the molecular weight of the sodium form of poliovirus is 2.6×10^6 daltons.

In contrast to extensive studies of the molecular weight of tobacco mosaic virus (TMV) ribonucleic acid [RNA (6, 7)], only limited physical data exist for the RNA molecules of animal viruses. Poliovirus RNA has been claimed to behave similarly to TMV RNA in the analytical ultracentrifuge (25); it has, therefore, been assumed that its molecular weight was approximately 2×10^6.

Measurement of the lengths of both single- and double-stranded RNA forms under the electron microscope did not corroborate this figure and led to a new estimation of the molecular weight of poliovirus RNA, which was in turn verified by sedimentation analysis.

MATERIALS AND METHODS

The Mahoney strain of poliovirus was grown in HeLa cells as described previously (4). The cells were washed with ice cold Earle's saline solution 7 hr after infection (10). Cytoplasmic extracts were prepared (12) and treated with 1% sodium dodecyl sulfate (SDS). These were centrifuged for 4 hr at 75,000 $\times$ *g*. The sedimented virus was suspended in SDS buffer [0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (*p*H 7.4), 0.1 M NaCl, 0.001 M ethylenediaminetetraacetate (EDTA), 0.5% SDS] and purified by centrifugation through a 28-ml gradient of 15 to 30% (w/w) sucrose in SDS buffer (Spinco model L_2, SW 25.1 rotor, 3 hr at 63,000 $\times$ *g*, 22 C). Viral RNA was extracted according to Mandel (22) by treatment of the purified virus with SDS at *p*H 4.1. SDS was then precipitated by addition of 0.1 M KCl in the cold. Viral RNA was further purified by centrifugation through a 28-ml gradient of 15 to 30% sucrose in 0.01 M Tris-hydrochloride (*p*H 7.4), 0.1 M KCl, 0.001 M EDTA (Spinco model L_2, SW 25.1 rotor, 18 hr at 60,500 $\times$ *g*, 5 C). Purified poliovirus RNA was then dialyzed against 0.02 M Tris-hydrochloride (*p*H 7.5) concentrated by dialysis against Ficoll (Pharmacia), and stored frozen in liquid nitrogen.

TMV grown in $^{14}CO_2$ was a generous gift from L. Hirth. The virus was treated with 20 μg of Pronase per ml (Calbiochem) for 40 min at 22 C, then mixed with an SDS-treated cytoplasmic extract from 10^8 HeLa cells. The mixture was extracted with phenol at 37 C. After precipitation with ethyl alcohol, the labeled RNA was further purified by sucrose gradient centrifugation in SDS buffer.

Poliovirus double-stranded RNA was prepared from HeLa cells which had been labeled with 20 μc/ml of tritiated uridine (22 c/mmole) in the presence of 5 μg of actinomycin D per ml. At 3.8 hr after infection, cytoplasmic extracts were prepared, and centrifuged for 40 min at 100,000 $\times$ *g* at 4 C. Resulting pellets were suspended in SDS buffer and precipitated with 2 M LiCl. Labeled double-stranded RNA was purified from the LiCl supernatant fractions by exclusion chromatography on 2% agarose (Sepharose 2 B, Pharmacia) according to Baltimore (3).

Samples for electron microscopy were prepared following the technique of Kleinschmidt (17, 18). Single-stranded RNA was first diluted with 8 M urea (15, 16). Double-stranded RNA was examined with and without addition of 8 M urea. Approximately 0.2 ml of a 1 M ammonium acetate solution, *p*H 8, containing from 1.7 to 4.0 μg of RNA per ml and 0.01% cytochrome *c* was allowed to flow down a clean inclined glass slide onto a hypophase of either 0.015 M ammonium acetate or 4 M urea. The resultant film was picked up on carbon coated Formvar films supported by 300 mesh grids. The specimens were dried in ethyl alcohol and shadowed with uranium oxide at an angle of 7°. Electron micrographs were made with a Philips EM 200 at a nominal magnification of 9,100 to 15,600 times; actual magnifications were determined and controlled with carbon grating replicas. Lengths of RNA molecules were determined on positive prints with a map measurer. As previously reported (16), grids rich in double-stranded RNA were more easily obtained than grids rich in single-stranded RNA.

RESULTS AND DISCUSSION

All poliovirus RNA molecules observed were linear, i.e., neither circular nor branched (Fig. 1a).

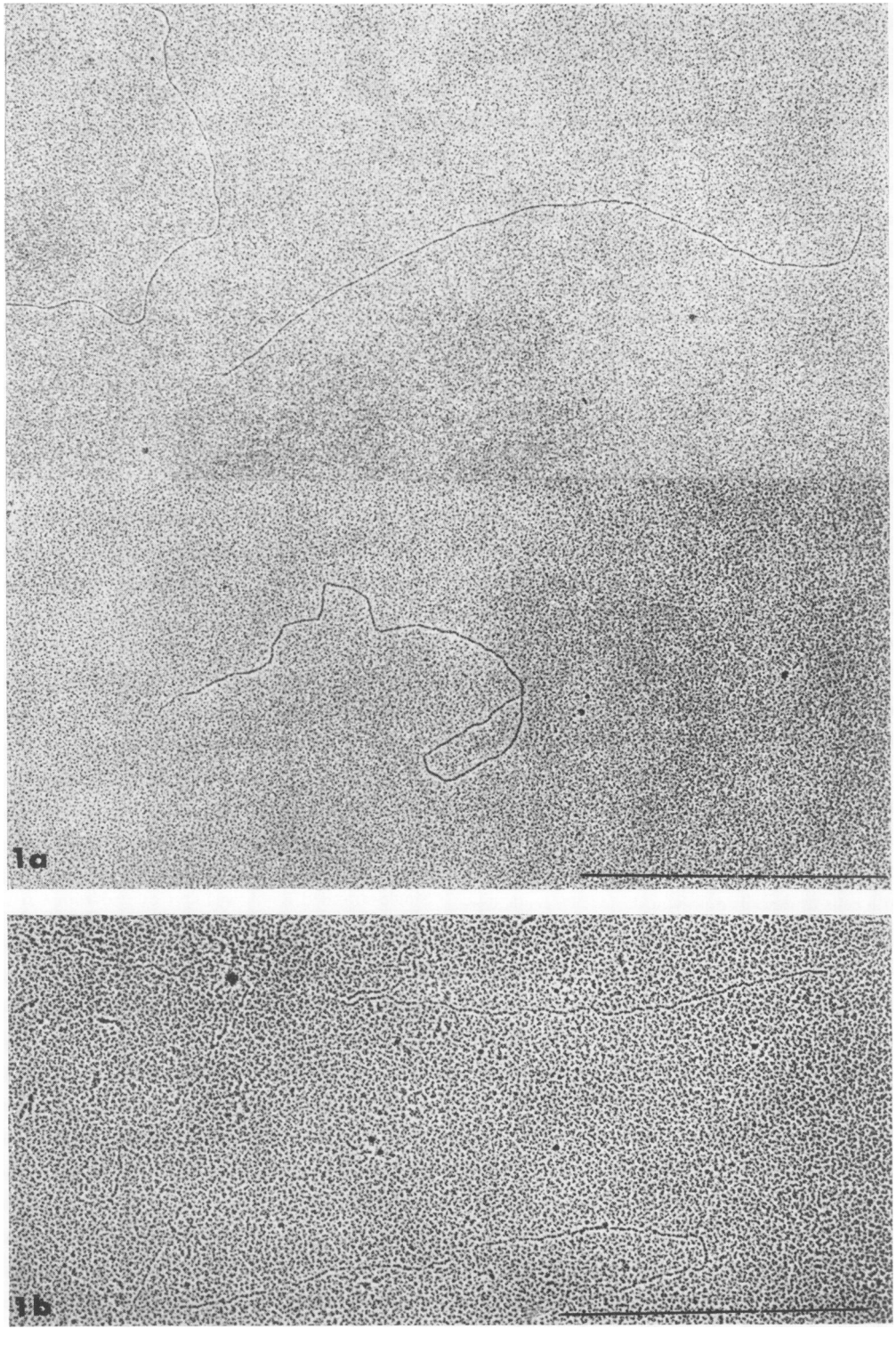

FIG. 1. *Poliovirus single- and double-stranded RNA molecules. (a) Single-stranded viral RNA diluted in 8 M urea and spread on ammonium acetate buffer; × 45,500. (b) Double-stranded RNA spread in the absence of urea; × 45,500.*

No differences in configuration or length were found between the molecules spread on ammonium acetate buffer and those spread on 4 M urea. Data obtained through both techniques were therefore pooled into one histogram (Fig. 2). The modal length of the 376 molecules of viral RNA examined is 2.40 μm. One half of these molecules have lengths between 2.05 and 2.75 μm, with a mean of 2.37 $\pm$ 0.13 μm. A second peak was found at 1.10 to 1.20 μm, which probably corresponds to half molecules.

All poliovirus double-stranded RNA molecules examined also had a linear configuration (Fig. 1b). As previously observed with the replicative form RNA molecules of both R17 bacteriophage (13, 14) and encephalomyocarditis (EMC) virus (N. Granboulan and L. Montagnier, *unpublished data*), addition of 8 M urea did not induce any change in morphology or length of poliovirus double-stranded RNA. Data obtained with and without 8 M urea were, therefore, pooled into the same histogram (Fig. 3). An analysis of 249 molecules reveals a modal length of 2.40 μm, with 51% of the molecules having lengths between 1.95 and 2.85 μm. Their mean length is 2.37 $\pm$ 0.14 μm.

Thus, single- and double-stranded molecules have the same length, as is the case for single- and double-stranded RNA molecules of R17 bacteriophage (13, 14) and EMC virus (Granboulan and Montagnier, *unpublished data*). This shows that elongation of single-stranded RNA is negligible under the conditions used in these studies. Since poliovirus RNA was examined under conditions identical to those used for R17 RNA, the value for internucleotide spacing determined for R17 RNA can be applied to the calculation of the total number of bases in poliovirus RNA. This value is 3.17 A [total number of bases in R17 RNA, 3,342 (26); measured length, 1.06 μm (13)]. Therefore, poliovirus single-stranded RNA should consist of 7,500 $\pm$ 400 nucleotides (a ratio of 23,700 $\pm$ 1,300 nucleotides to 3.17 μm). The average molecular weight per nucleotide is 322 daltons [as calculated from the reported base compositions (2, 5)]. It follows that the molecular weight of poliovirus RNA should be approximately 2.42 $\times$ 10^6 daltons (or about 2.6 $\times$ 10^6 daltons for its sodium form).

As previously discussed (16), internucleotide spacings determined by X-ray diffraction [3.05 A according to Langridge and Gomatos (19), or 2.73 A according to Arnott et al. (1)] do not seem to apply to RNA molecules spread for electron microscopy. In any event, they would yield even higher values for the molecular weight of poliovirus RNA, since the total number of nucleotides in poliovirus RNA would then be 7,800 or 8,700, respectively.

The value of 2.6 $\times$ 10^6 daltons for the Na^+ form of poliovirus RNA fits well with that calculated on the same basis for EMC virus RNA

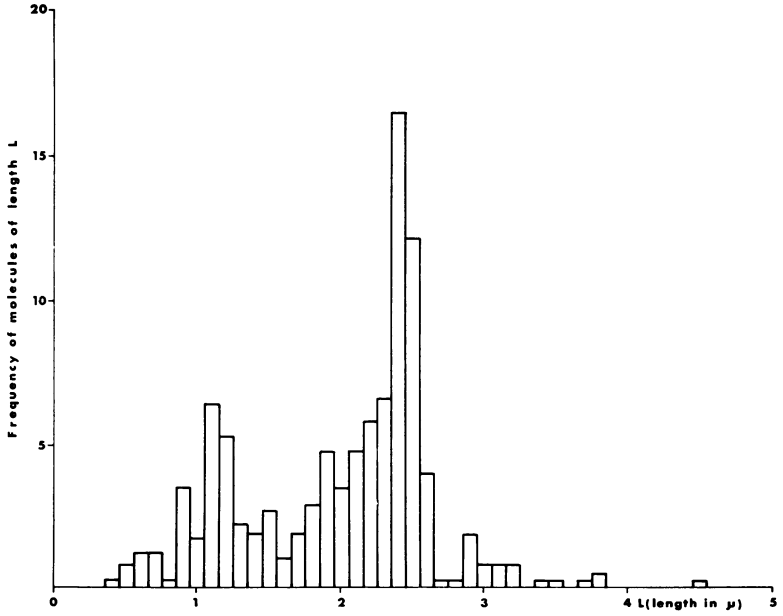

FIG. 2. *Length distribution of poliovirus RNA spread in the presence of urea. A total of 376 molecules was analyzed.*

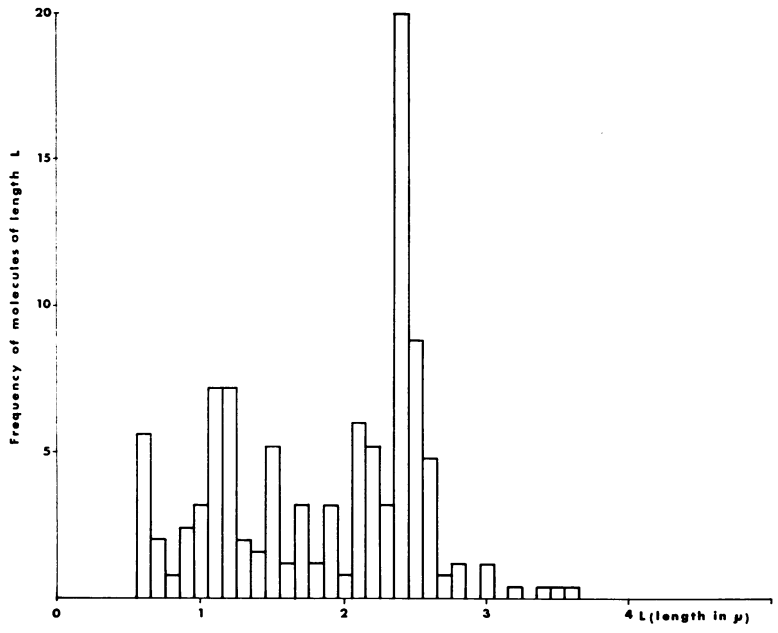

FIG. 3. *Length distribution of poliovirus double-stranded RNA. A total of 249 molecules was analyzed.*

(2.7×10^6 daltons, Granboulan and Montagnier, *unpublished data*). It should be noted that a molecular weight of close to 3×10^6 daltons had been assigned to EMC virus RNA from the results of ultracentrifugation studies (24). McGregor and Mayor (21) reported that the length of the poliovirus nucleoproteinic strands released from purified virions by heat treatment was 2.0 μm, and, thus, concluded that the molecular weight of poliovirus RNA was 2×10^6. The discrepancy between their results and ours could be due to the absence of deproteinization of the RNA in their experiments.

To check the value obtained from electron microscopy measurements, the sedimentation coefficient of poliovirus RNA was determined. Previous studies had indicated that this coefficient was approximately 35*S* in 0.1 M salt (9). A sample of poliovirus RNA identical to that used for the electron microscopy measurements was examined in a Spinco analytical ultracentrifuge after dilution with 0.05% SDS buffer. A value of approximately 33*S* was found (*unpublished data*). A comparison was then made of the sedimentation patterns in sucrose gradients of poliovirus RNA and TMV RNA. Sedimentation coefficients, determined according to Martin and Ames (23), and taking 28*S* HeLa cells ribosomal RNA as reference, were approximately 27*S* for TMV RNA and 33*S* for poliovirus RNA (Fig. 4). Although the precision of these absolute numbers is somewhat limited, the important fact is that poliovirus RNA sedimented about 1.2 times faster than TMV RNA. Assuming they have

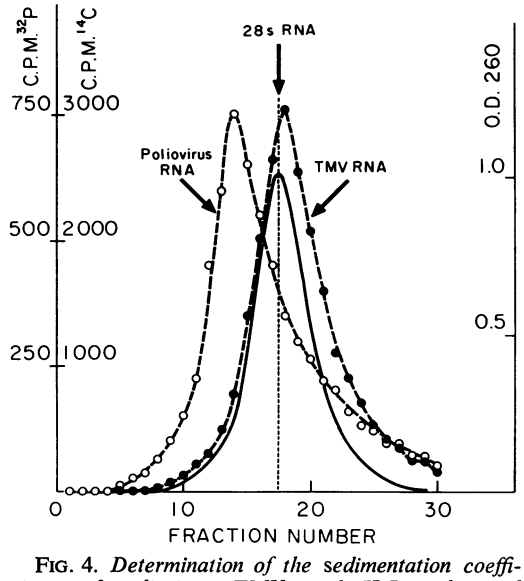

FIG. 4. *Determination of the sedimentation coefficients of poliovirus, TMV, and HeLa ribosomal RNA* molecules. *^{14}C labeled TMV RNA* (●), *^{32}P-labeled poliovirus RNA* (○), *and 28S ribosomal RNA (bar) were mixed and centrifuged for 17 hr at 48,000 × g at 22 C through a 28-ml gradient of 15 to 30% (w/w) sucrose in SDS buffer (Spinco model L_2, SW 25.1 rotor). Only the first 30 fractions of the gradient are shown.*

identical hydrodynamic properties, it follows from the Mandelkern-Flory equation (8, 11) that the molecular weight of poliovirus RNA should be 1.3 times greater than that of TMV RNA.

Taking the latter as 2×10^6 daltons (6, 7), an approximate value of 2.6×10^6 daltons is found again for the Na^+ form of poliovirus RNA. A similar calculation, based on a molecular weight of 1.9×10^6 for 28*S* ribosomal RNA (20), would assign poliovirus RNA a molecular weight of 2.45×10^6.

Thus, poliovirus RNA should contain the information for approximately 2,500 $\pm$ 130 amino acids. Assuming the average molecular weight per amino acid is 110 (known values are 100 in hemoglobin, 107 in f2 coat protein, and 113 in lysozyme), it follows that poliovirus RNA could code for the synthesis of proteins with a total molecular weight of about 275,000. Also, from the known data concerning reticulocyte polyribosomes (27), it can be estimated that the average number of ribosomes which could be accommodated on a strand of 7,500 nucleotides is 80. The reason why there seem to be only 40 ribosomes per poliovirus RNA molecule in viral polyribosomes (28) is unknown.

ACKNOWLEDGMENTS

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